THE EFFECT OF GRADED LEVELS OF DIETARY STARCH ON CECAL ENVIRONMENT IN HORSES

A Thesis

by

KRISTEN LEIGH WILSON

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2009

Major Subject: Animal Science

THE EFFECT OF GRADED LEVELS OF DIETARY STARCH ON CECAL ENVIRONMENT IN HORSES

A Thesis

by

KRISTEN LEIGH WILSON

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Approved by:

Chair of Committee,	Josie Coverdale
Committee Members,	Robin Anderson
	David Hood
	Brett Scott
Head of Department,	Gary Acuff

May 2009

Major Subject: Animal Science

ABSTRACT

The Effect of Graded Levels of Dietary Starch on Cecal Environment in Horses.

(May 2009)

Kristen Leigh Wilson, B.S., University of Georgia

Chair of Advisory Committee: Dr. Josie Coverdale

Eight cecally fistulated geldings were used in a randomized 4 x 4 Latin square design to observe the effect varying levels of dietary starch had on cecal environment. The 4 treatment rations contained 2 g starch/kg BW (Diet 2), 4 g/kg BW (Diet 4), 6 g/kg BW (Diet 6), or 8 g/kg BW (Diet 8). The rations were comprised of a commercial pelleted feed to meet 2 g starch/kg BW in each treatment, with ground corn used to fulfill the remaining starch requirements in each diet. Soybean meal was added to ensure diets were iso-nitrogenous, and cottonseed hulls were used to equalize dry matter intake. A 21 day adaptation period was allowed before cecal contents were sampled. Samples were drawn 4 hours after the morning meal and were immediately tested for pH. Samples were used to count total anaerobic bacteria and lactic acid bacteria, as well as determine methane activity, ammonia activity, volatile fatty acids, and in vitro dry matter digestibility (IVDMD). Stoichiometric calculations were performed to give an indirect measure of fermented hexose, methane, and carbon dioxide.

Diet did not influence dry matter intake (DMI), however it did have an effect on starch intake ($P < 0.0001$) and caused a linear increase in starch consumption as the

amount of offered starch increased ($P < 0.0001$). Diet did not influence the pH of the cecum ($P > 0.05$), although a tendency for a linear decrease ($P < 0.06$) in pH from 6.92 – 6.58 occurred when dietary starch increased. Total anaerobic bacteria and lactic acid bacteria were unaffected by treatment diets ($P > 0.05$). Propionate production was affected by dietary treatment ($P < 0.05$), causing a quadratic increase ($P = 0.04$) from 8.26 to 14.13 mM as starch in the diets increased. Diet did not affect the production of acetate, butyrate, or ammonia ($P > 0.05$). Results found that stoichiometric calculations and IVDMD values were not affected by diet ($P > 0.05$). These results show that starch intake influenced the production of fermentative by-products, which caused decreases in pH, although there was no observed increase in the bacterial populations of the cecum.

DEDICATION

This thesis is dedicated to my family who has always been there to love and support me. To my father, who has given me strength and wisdom in all of my pursuits no matter where they may lead. To my sisters, who can always find a way to make me laugh no matter how stressed out I may be. And to my mother, who was so proud of me for taking this chance, and who I wish was still here to see me finish it.

ACKNOWLEDGEMENTS

I would like to thank my committee chair, Dr. Coverdale, and my committee members, Dr. Anderson, Dr. Hood, and Dr. Scott for their guidance and support throughout the course of this research.

Thanks also go to all of the grad students in the equine science department for their help and friendship over the course of this project. I would also like to thank Jackie Kotzur and Nathan Krueger at USDA for their help and guidance with analyzing samples in the lab. Furthermore, thanks go to Cargill for providing the SafeChoice used in this study.

Finally, thanks to my father and sisters for their love and encouragement, without you none of this would have been possible.

TABLE OF CONTENTS

CHAPTER Page

LIST OF FIGURES

LIST OF TABLES

CHAPTER I

INTRODUCTION AND REVIEW OF LITERATURE

Introduction

The use of cereal grains have become common practice in providing for the increased nutritional needs in the modern performance horse. These horses are typically fed large concentrate meals that are high in non-structural carbohydrates (NSCs), such as starch, and low in fiber that is typically found in grasses and hays. The horse, however, has a digestive tract that was adapted to handle multiple small meals per day of high fiber forages, possessing a short small intestine and a large cecum to allow for fermentation of fiber. Because of this, the digestive tract of the horse is not always capable of handling large amounts of concentrate, and can sometimes lead to digestive upsets such as acidosis, colic, or laminitis (Garner et al., 1975). These problems have been linked to the small intestine being overwhelmed when large amounts of NSCs are fed, allowing them to pass through to the large intestine for microbial fermentation (Garner et al., 1975; Bailey et al., 2002). The first portion of the hindgut the digesta reaches is the cecum, the primary site of microbial fermentation in the horse. While the affect of concentrate diets has been extensively researched in food animals such as ruminants, this area is lacking with respect to equine nutrition. Although the cecum is similar to the rumen of ruminant species, the positioning of the rumen in the foregut of the animal prevents equine researchers from relying solely on this data to predict a response in the horse.

This thesis follows the style of Journal of Animal Science.

Therefore, the importance of the cecum, the bacteria that reside there, and how NSCs affect cecal environment are necessary areas of research to ensure that horses are being fed properly and to avoid digestive diseases.

Cecum

The segment of the large intestine, known as the cecum, is a 1.22 m long blind sac located at distal end of the ileum with a capacity of approximately 25 to 30 L (Argenzio et al., 1974a). The cecum represents 16% of the total gastrointestinal tract volume in the horse, which is greater than that of both ruminant and other non-ruminant species such as cattle (3%) and pigs (6%) (Pond et al., 1995). This portion of the large intestine is comprised of four longitudinal bands, called *taeniae caeci*, that are situated on the dorsal, ventral, left, and right sides of the cecum (Ellis and Hill, 2005). Digesta enters the cecum through a valve at the junction between the ileum and cecum known as the ileo-caecal valve and exits the cecum through the caeco-ventral colonic valve (Argenzio, 1975). According to Sellers and Lowe (1986), digesta is moved through the cecum by peristaltic waves that push the contents from the cecal tip to the top of the cecum. The movement is soon followed by waves moving distally, forcing digesta back in the direction of the cecum tip (Sellers and Lowe, 1986). The constant movement in the cecum allows for the mixing of the digestive contents and eventual entrance to the right ventral colon (Sellers and Lowe, 1986).

The cecum of the horse is often compared to the rumen of cattle and other ruminant species due to its function and environment. However, the major difference between these two digestive structures is that in ruminants, digesta first enters the rumen

and then passes through the remainder of the intestinal tract. In contrast, the equine cecum receives digesta after it has been exposed to the digestive processes of the stomach and small intestine. The stomach and small intestine are primarily responsible for the digestion and absorption of proteins and non-structural carbohydrates (NSCs), leaving structural carbohydrates, such as cellulose and hemicelluloses, to be degraded by the microbial populations in the cecum. The diet of the feral horse consists of multiple small meals with very little non-structural carbohydrates, and the bulk comprised of fibrous forages. This type of meal is well suited to the horse's small and relatively simple stomach which is followed by a short small intestine (Pond et al., 1995). The horse adapted this prececal digestive physiology because the small meals they ingest do not require a large stomach to retain digesta, while a short small intestine would suffice to digest and absorb NSCs. After ingestion of a meal, digesta remains in the stomach for approximately 2 hrs and quickly passes through the small intestine to appear in the cecum as soon as 3 hrs after feeding (Argenzio et al., 1974a). Upon entrance to the cecum, digestible material in the cecum remains approximately 2.9 hrs before moving to the right ventral colon (Miyaji et al., 2008). The retention time in the cecum can vary, however, with large particles remaining in the cecum for up to 48 hrs, while small particles have been observed to reach the right ventral colon within 2 hrs (Argenzio et al., 1974a). The longer retention time in the cecum allows the bacteria in the cecum more time to ferment digesta particles, while the smaller particles have a greater surface area and can be fermented faster. The rate of passage and mean retention time of the digesta are therefore important to the functions of the cecum.

The cecum preserves a nearly anaerobic environment which allows microbial populations that require/tolerate very low oxygen to reside in the large intestine (Argenzio, 1975). The temperature in the cecum remains similar to the body temperature of the horse, from 37° to 38° Celsius. This portion of the large intestines maintains an average pH of 6.7; however, this number can vary largely depending on the horse and the diet available to the animal (Mackie et al., 1988). The cecum is capable of absorbing products created by the microbes, such as volatile fatty acids (VFAs), which allow it to sustain a near neutral pH (Annison and Lewis, 1959). Unlike the small intestine, there are no digestive secretions in the cecum. Products secreted into the cecum include HCO_3^-, which acts as a buffer in the large intestine and mucus (Argenzio, 1975). The combination of digesta retention and cecal environment are important to the primary functions of the equine cecum: microbial fermentation, absorption of fermentative by-products, and water absorption.

The cecum of the horse is populated with a wide variety of bacteria and protozoa that serve the function of fermenting material that is not digested or absorbed in the small intestine. The substrates that reach the hindgut are those that are not able to be digested and absorbed prececally, such as structural carbohydrates. However, components that would normally disappear prececally can reach the cecum if fed at too high a rate. Examples of this are NSCs, which can overwhelm the capacity of the small intestine if fed at 2 – 4 g per kg BW (Potter et al., 1992; Meyer et al., 1995). Bacteria located in the cecum are capable of degrading the ß-1,4 linked polymers of cellulose that are present in the cell walls of forages (Frape, 2004). The digestive enzymes in the

small intestine are unable to break the ß-1,4 bonds, causing the horse to rely on the cecal bacteria to digest these components. The bacteria responsible for the degradation of these fibrous materials are referred to collectively as cellulolytic bacteria. Amylolytic bacteria are the starch-fermenters of the cecum, degrading NSCs through the use of α-amylases and fermenting the glucose that is released. Proteolytic bacteria are capable of fermenting protein that escapes digestion in the stomach and small intestine and represent 19.7% of the bacteria in the equine cecum (Kern et al., 1973). The variety of substrates reaching the cecum allows for bacteria to produce volatile fatty acids (VFAs) as well as other products such as methane, carbon dioxide, and ammonia.

The absorption of VFAs and other by-products of fermentation constitute another important function of the cecum. The primary VFAs that are produced through microbial fermentation: acetate, butyrate, propionate (Hungate, 1966). The types of VFAs produced in fermentation are correlated with the type of substrate present in the cecum. Microbes in the cecum produce 40 – 50 mM of VFAs, with acetate representing 70%, propionate 20% and butyrate 8% (Hintz et al., 1971). Studies performed in both equines and ruminant species have found that diets high in fiber produce greater concentrations of acetate, while animals fed high starch diets produce more propionate (Annison and Lewis, 1959; Hintz et al., 1971). The buildup of these VFAs can cause a decrease in the pH of the cecal chyme, which makes the ready absorption of these by-products essential to maintaining a hospitable environment for microbes (Hungate 1966). This absorption of VFAs is often coupled with the movement of electrolytes across the lumen of the large intestine. When the pH of the cecal contents is similar to the pK

value of a VFA, passive absorption occurs along with a net absorption of NaCl (Frape, 2004). The VFAs, once entering the bloodstream, constitute 60 to 70% of the horse's body energy needs through a variety of metabolic functions (Argenzio et al., 1974b). Acetate can be converted into acetyl-CoA used for energy by participating in the citric acid cyle at the citrate synthesis step (Frape, 2004) or can proceed into fatty acid synthesis (Annison and Lewis 1959). Butyrate can also be used for energy by converting into acetoacetate ß-hydroxybutyrate, which can then become acetyl-CoA (Frape, 2004). Butyrate serves as the preferred energy source for intestinal epithelial cells, making it an important factor in the health of the intestinal tract. Propionate can also be used as an energy substrate when it becomes succinyl-CoA and is inserted into the citric acid cycle (Frape, 2004). Propionate can alternatively be used in the production of glucose through gluconeogenesis. The absorption of these VFAs through the cecum not only provides energy for the horse, it also serves as an important mechanism for the absorption of water.

The large intestine of the horse, on average, contains 75% of the total gastrointestinal water (Argenzio et al., 1974a). The cecum itself receives approximately 19.4 l of water from the ileum of the small intestine and absorbs 13.5 l per day (Argenzio et al., 1974a). The remaining water passes through the large intestinal tract, with only 1.5 liters being excreted in the feces (Argenzio et al., 1974a). A large portion of the horse's electrolytes are also absorbed in the large intestine, along with water. As previously mentioned, NaCl is absorbed along with VFAs and water, approximately 96% of the sodium and chloride that enters the cecum from the ileum is absorbed as well

as 75% of the soluble potassium and phosphate (Frape, 2004). This movement of water and electrolytes is essential to the absorption of VFAs and to maintaining a constant pH in the cecum, which allows a variety of microbial populations to flourish in the environment.

Microbial Populations of the Cecum

The cecum's function as the site of microbial fermentation is an integral part of the horse's digestive tract, but it is also an area lacking in research. The cecum of the horse is very similar to the rumen, except for its placement in the hindgut while the rumen is located in the foregut. This arrangement prevents equine researchers from being able to directly apply the wealth of ruminant data to horses since all equine digesta is at least partially digested or absorbed prececally. However, similarities in their microbial populations are noted between the two digestive organs. Research by Kern et al. (1973) and Slyter et al. (1970) observed that the rumen of cattle and the cecum of the horse are inhabited by similar bacterial populations with variations being found in the total number of bacteria. Kern et al. (1974) found that the rumen of steers had, on average, 3150×10^7 bacteria/g of ingesta, while the cecum of ponies contained 642×10^7 bacteria/g of ingesta. This indicated that the rumen contains a more dense bacterial population than the equine cecum, and allows ruminants to more thoroughly digest feedstuffs (Kern et al., 1974). Although knowledge of the microbial populations in the equine cecum may not be as extensive as that of the rumen, the identification of many of the primary populations have been determined and classified into the following categories: cellulolytic, proteolytic, or amylolytic bacteria and protozoa.

Cellulolytic Bacteria. Cellulolytic bacteria are responsible for the degradation of structural carbohydrates such as cellulose, hemicellulose and pectin. These bacteria range in total population from 1.8×10^4 to 3.04×10^7 bacteria/g of ingesta, approximately 100-fold lower than ruminants (Hungate 1966; Kern et al., 1973; Kern et al., 1974; Mackie et al., 1988; Moore et al., 1993; Goodson et al., 1998; Julliand et al., 1999). Depending on the diet, these bacteria can represent 3 – 9% of the total bacteria in the cecum (Kern et al., 1974; Julliand et al., 1999). Horses that receive fiber-rich diets tend to have larger populations of cellulolytic bacteria, while low-fiber diets will have a lower concentration of these bacteria due to decreased availability of usable substrates. Although a wide variety of cellulolytic bacteria exist, 3 predominate species have been identified in the cecum: *Ruminococcus flavefaciens*, *Ruminococcus albus*, and *Fibrobacter succinogenes* (Julliand et al., 1999).

The first species, *R. flavefaciens*, was determined by Julliand et al. (1999) to be the predominant species of cellulolytic bacteria in the equine cecum. This Gram-positive bacterium uses an enzyme complex known as a cellulosome to cleave off portions of a cellulose molecule, until only glucose remains. These complexes use endo-β-1,4-glucanase to produce reducing and non-reducing ends on the cellulose chain by hydrolyzing the β-1,4 bonds of cellulose. The non-reducing ends are then attacked by exo-β-1,4 glucanase, which cleaves off molecules of cellobiose that can be hydrolyzed by β-glucosidases to produce glucose (Doerner et al., 1990; Gardner et al., 1987; Pettipher et al., 1979). Glucose molecules then enter the Embden-Meyerhof pathway, yielding 2 molecules of pyruvate. The pyruvate molecules go through anaerobic

fermentation, producing acetate through oxidative decarboxylation (Hungate, 1966). Additionally, ethanol can be produced during this conversion, and is quickly absorbed by the horse. Formate is also produced during fermentation, and acts as a hydrogen source for the production of methane (Stainer et al., 1986). Another *Ruminococcus* species, *Ruminococcus albus* has a similar fermentation pathway, and has also been determined to be another of the major cellulolytic species in the horse.

Ruminococcus albus is a species of cellulolytic bacteria commonly found in ruminants, but has also been detected in the cecum of horses (Hungate, 1950; Julliand, 1999). Although the population of *R. albus* in the cecum is small (Julliand et al., 1999; Daly et al., 2001), it plays an important role in the fermentation of cellulose. *R. albus* uses a cellulosome complex similar to that of *R. flavefaciens* to break down cellulose yielding glucose (Hungate, 1966). The fermentation of glucose molecules in the rumen yield products such as acetate, ethanol, and formate (Miller and Wolin, 1973). The products of equine strains of *R. albus* have yet to be determined, but are believed to be similar to rumen strains. Because *R. albus* is not densely populated in the equine cecum, research is often performed on larger bacterial populations, such as *F. succinogenes*.

Another predominant species in the equine cecum is *Fibrobacter succinogenes* (Davies, 1964; Julliand et al., 1999; Lin et al., 1995). This species was first isolated and classified in the rumen as *Bacteroides succinogenes* (Hungate, 1950), and was later placed in its own genus *Fibrobacter* by Montgomery et al. (1988). A bacterium similar to the one classified by Hungate (1950) was also isolated in the cecum of horses by Davies in 1964 and was more recently found to represent 12% of the total rRNA when

cecal contents were analyzed using taxon-specific probes (Lin et al., 1995). This Gram-negative rod uses endoglucanases and cellobiases to completely degrade cellulose into glucose, which can then be fermented into acetate and succinate (Miller, 1978; Gorleau and Forsberg, 1981; Forsberg, 1997). Although *F. succinogenes* has an important role in the fermentation of cellulose, along with *R. flavefaciens* and *R. albus*, there are still a number of other bacteria in the cecum that also play a role in the breakdown of fiber.

Julliand et al. (1999) determined the primary cellulolytic species in the cecum; however, there are still a wide variety of bacteria that play a similar role, despite not having as large of a population. Daly et al. (2001) observed that 37% of the sequences recovered represented *Clostridium* spp., *Butyrivibrio* spp., and *Eubacterium* spp., all of which participate in cellulose degradation. The cellulolytic *Clostridium* species also use cellulosomes to breakdown cellulose and produces acetate, butyrate, and formate through fermentation (Hungate, 1966). Another cellulolytic species, *Butyrivibrio*, are capable of degrading a variety of substrates including cellulose, hemicelluloses, and starch (Hungate, 1966). One specific strain, *Butyrivibrio fibrisolvens*, has been found to be more efficient at degrading hemicelluloses rather than cellulose, while producing butyrate, formate, and acetate as fermentative by-products (Davies, 1964; Dehority et al., 1967; Stewart and Bryant 1988). *Eubacterium* spp. has been detected in both ruminants and horses, producing formate, butyrate, and lactate from cellulose molecules (Stewart and Bryant; Daly et al., 2001). Even though cellulose plays a significant role in providing energy substrates for the animal, the breakdown of cell wall components is also important to ensure fibrous forages are being thoroughly utilized.

The degradation of other cell wall components, hemicelluloses and pectin, can also be performed by bacteria in the cecum. A few previously mentioned bacteria, *F. succinogenes* and *B. fibrisolvens*, are capable of degrading hemicelluloses and pectin, but other species of bacteria and protozoa have also been found to perform this action. Mackie and Wilkins (1988) determined that 3.6×10^8 bacteria/gram of ingesta are capable of degrading hemicelluloses in the cecum of the horse. The bacteria use glycanhydrolases, glycosidases, and β-galactosidase to convert hemicelluloses molecules into xylose, arabinose, and galactose (Bonhomme-Florentin, 1988). The xylose and arabinose released in this reaction can be incorporated into the pentose phosphate pathway as xylulose-5-phosphate (Prins, 1977), while galactose may be fermented into acetate and ethanol (Thomas et al., 1980). Two protozoa found in the cecum, *Cycloposthium* spp. and *Belpharocorys* spp., have been found to play a role in the degradation of pectin, using polygalacturonase, pectin lyase, and pectinesterase to break pectin down into galacturonic acids, oligouronides, and methanol (Bonhomme-Florentin, 1988). Galacturonic acid and oligouronides can be fermented into acetate and propionate, while methanol can be incorporated into methanogenesis in ruminants, but there has been no research as to how these products are incorporated by the horse (Hungate, 1966; Pol and Demeyer, 1988). These cellulolytic bacteria play an essential role in the horse by digesting dietary components that the horse is unable to degrade on its own. However, even portions of the diet that the horse does possess enzymes for can reach the cecum to be fermented by the populations there; such is the case with proteins

which may then be fermented by microorganisms in the cecum known as proteolytic bacteria.

Proteolytic Bacteria. Proteolytic, or protein fermenting, bacteria represent approximately 19.7% of the bacteria in the equine cecum and includes a variety of different bacteria (Kern et al., 1973). Kern et al. (1973) identified a number of bacteria with proteolytic activity, such as *Streptococcus bovis, Bacteroides amylophilus,* and *Bacteroides ruminicola*. How these bacteria specifically degrade protein, however, has yet to be determined. The reason for this difficulty is because proteolytic bacteria work cooperatively to denature the complex protein structures through the use of proteases and peptidases (Wallace et al., 1997). The product of this degradation is the release of peptides, with the small di- and tri- peptides converted into VFAs, while large peptides are incorporated into microbial crude proteins (Wright and Hungate, 1967). Ruminants are capable of utilizing these bacterial proteins through digestion and absorption in the small intestine. The location of microbial fermentation in the horse, however, occurs after the small intestine, preventing the horse from utilizing microbial crude protein, resulting in fecal losses (Frape, 2004). However, the horse is able to utilize other products of fermentation such as ammonia and VFAs, which are produced when amino acids are fermented in the hindgut (Reitnour and Salsbury, 1972; Wysocki et al., 1975). Microbes in the cecum are also use these products as a source of nitrogen to aid in growth and productivity (Frape, 2004).

Amylolytic Bacteria. Amylolytic bacteria are responsible for the degradation of any starch which may reach the large intestine. Due to the low capacity of the small

intestine and relatively low activity of enzymes, this occurs more frequently with modern equine diets that rely on cereal grains to meet the energy needs of horses. These amylolytic bacteria represent 73.1% of the total bacteria in the equine cecum when the horse is fed a forage-only diet, with this number increasing to 92% when concentrate was added to the diet (Goodson et al., 1988). The increase in population can be attributed to the availability of starch in concentrate diets. This ability of the hindgut to ferment starch, coupled with prececal digestion and absorption, allows the horse to have near 100% digestion of dietary starch (Arnold et al., 1981). Two starch-utilizing bacteria, *Streptococcus bovis* and *Lactobacillus* spp. were first identified in the equine large intestine by Alexander and Davies (1963) and have since then been labeled as the predominate amylolytic species in the cecum (Kern et al., 1973; Garner et al., 1978; Al Jassim et al., 2005).

Streptococcus bovis, a Gram-positive cocci has since been identified in the gastrointestinal tract of many species including ruminants, horses, pigs, rabbits and guinea pigs (Alexander and Davies, 1963). In the horse, populations of *S. bovis* have been found to range from 5×10^5 to 2×10^8 bacteria/g ingesta, with the number increasing as concentrate is added to the diet (Alexander and Davies, 1963; Kern et al., 1973; Julliand et al., 2001). These bacteria are able to tolerate the occasional appearance of oxygen as well as a lower pH, allowing them to quickly proliferate in not only the gastrointestinal tract, but in cultures as well (Hungate, 1966; Latham et al., 1979). The bacteria use an enzyme similar to α-amylase which cleaves the α-1,4 linkages of starch to produce molecules of glucose, much like the natural enzymes found in the small

intestine (Hungate, 1966). These molecules of glucose then go through homofermentation by *S. bovis,* generating lactate as the sole product of fermentation (Stainer et al., 1986). Homofermenters perform this act by utilizing the Embden-Meyerhof pathway to convert glucose into 2 pyruvate molecules. The bacteria then use lactate dehydrogenase to convert these pyruvate molecules into lactic acid (Stainer et al., 1986). Lactate molecules are then incorporated into the acrylate pathway, which uses the carbon skeleton of lactate to synthesize propionate. Another starch-utilizing bacterium, *Lactobacillus* spp, uses a similar method to ferment starch that may reach the hindgut.

Lactobacillus spp. is a Gram-positive rod representing the other major amylolytic bacteria in the equine cecum. This starch-digesting species can populate the cecum at 10^5 to 10^7 bacteria per g ingesta, and includes strains such as *Lactobacillus mucosae, Lactobacillus salivarius,* and *Lactobacillus reuteri* (Kern et al., 1973; Julliand et al., 2001; Bailey et al., 2003; Al Jassim et al., 2005). Similar to *S. bovis*, this species is typically a homofermenter, utilizing glucose molecules in the cecum to produce lactic acid. The molecules of lactate can then enter the acrylate pathway to produce propionate (Stainer et al., 1986). Heterofermentative species of *Lactobacillus* spp. exist, which are capable of producing ethanol in addition to lactate, but these strains have yet to be identified in horses (Stainer et al., 1986). The lactate produced by the homofermentative bacteria can act as a substrate for other species of microorganisms as well; these bacteria are referred to as lactic acid-utilizing bacteria.

Lactic acid utilizing bacteria are important to the health of the horse by removing lactate from the cecal environment, preventing a drop in pH. Alexander (1952) first detected these bacteria in the large intestine of the horse, and have now been found to represent 26.1% of the bacteria in the cecum (Goodson et al., 1988). The first species of these bacteria identified in the horse was *Veillonella* spp. (Alexander and Davies, 1963), and was later verified by Al Jassim et al. (2002) through PCR analysis. *Veillonella* spp. is capable of fermenting lactate through a randomizing succinate pathway, resulting in the production of both acetate and propionate (Stainer et al., 1986). Further research is needed to determine what other species exist in the equine cecum utilize lactate, this could play an important role in the uptake of lactate in order to prevent gastrointestinal upsets such as lactic acidosis. Another category of microorganisms that could use further research are protozoa, whose ultimate role in hindgut fermentation has yet to be determined.

Protozoa. There is very little information about the protozoan population in the equine cecum, although it does appear to have some role in nutrient digestibility. A study evaluating the effect of defaunation on the cecum noted that DM digestibility decreased slightly when protozoa no longer populated the cecum (Moore and Dehority, 1993). Further research has determined that protozoa in the equine cecum play a role in the fermentation of pectin, possibly explaining the decreased DM digestibility observed with defaunation (Bonhomme-Florentin, 1988). Despite the effect on dry matter digestibility, the removal of protozoa from the cecum found no change in digestibility of cellulose specifically (Moore and Dehority, 1993). Because protozoa seem to have very

little overall affect on digestibility, there has not been significant research to determine what affect these microbes may play on other substrates in the cecum. Bacteria and protozoa, together, play an important role in utilizing the variety of dietary components that enter the cecum of the horse. However, the balance of these microorganisms can be easily disturbed due to changes in the horse's diet, such as the inclusion of cereal grains. This can result in changes not only to the population of bacteria, but to fermentation by-products and the pH of the cecum.

Influence of Diet on the Cecal Environment

The addition of concentrate has been observed to cause a number of changes to the environment of the cecum of the horse due to the large amounts of NSCs found in cereal grains. Fluctuations of bacteria populations occur when NSCs, such as starch, become available as a substrate, resulting in increases in total population and changes to the predominance of the functional groups of cecal bacteria (Kern et al., 1973). These changes are then reflected in alterations to the products of fermentation, such as available volatile fatty acids, methane and ammonia, which can elicit changes to the pH of the cecum (Hintz et al., 1971; de Fombelle et al., 2001).

Changes to Microbial Populations. The effect of concentrate feeding on microbial populations was noted early in ruminant species; researchers observed an increase in total populations, and fluctuations in numbers and species of bacteria cultured from rumen fluid that had been exposed to higher amounts of concentrate (Hungate et al., 1952; Bryant and Burkey, 1953; Bauman and Foster, 1956; Maki and Foster, 1957). Total populations of rumen bacteria increased 2 to 3 times in animals fed

concentrate over those fed forage alone (Maki and Foster, 1957). This increase has been attributed to the presence of NSCs found in cereal grains allowing bacterial populations to flourish, with similar responses noticed in the cecum of equines. When horses fed hay-only diets were supplemented with concentrate, an increase in the total population of bacteria in the cecum from 10^7 to 10^9 bacteria/g digesta was observed (Kern et al., 1973; Julliand et al., 2001; Medina et al., 2002). These reactions can also occur when concentrate feeds are abruptly added to the diet and has been observed in both the rumen and the cecum. Grubb and Dehority (1975) witnessed an increase in total bacterial populations in the rumen of sheep when diets were changed from a forage-only diet to a high concentrate diet. Goodson et al. (1988) elicited a similar response in horses when diets were suddenly changed from a hay-only diet to a concentrate-only diet, with populations rising from 5.28×10^9 to 30.07×10^9 bacteria/g of digesta over a 24 hr period. The increase in total population observed with the addition of cereal grains has been attributed to the proliferation of amylolytic bacteria, specifically *Streptococcus bovis* and *Lactobacillus* spp.

Early studies in ruminant microbiology noticed that amylolytic species represented an increasing proportion of the rumen microflora when concentrate feeds were added to the diet (Hungate et al., 1952; Bryant and Burkey, 1953). *Streptococcus bovis* and *Lactobacillus* spp. were some of the first bacteria cultured in both horses and ruminants when concentrate was added to the diet, as well as the most prolific (Hungate et al., 1952; Kern et al., 1973). Species of *Streptococcus* and *Lactobacillus* have been observed to increase in the cecum when concentrate was gradually added to the diet by

Julliand et al. (2001). The study saw *Lactobacilli* spp. increase from 4.17×10^5 to 7.34×10^6 bacteria/mL and *Streptococci* spp. increased from 5.00×10^5 to 8.94×10^6 when horses were switched from an all-hay diet to 50:50 hay:barley diet (Julliand et al., 2001). Medina et al. (2002) also observed increases in lactic acid bacteria when comparing horses fed a high fiber diet to those on a high starch diet. *Streptococcus* spp. populations increased from 2.51×10^6 to 5.01×10^7 cfu/mL, while *Lactobacilli* went from 3.9×10^6 to 3.17×10^7 cfu/mL when the horses were fed the starch diet compared to the high fiber diet. The abrupt incorporation of concentrate into the equine diet has also resulted in an increase of lactic acid bacteria. Goodson et al. (1998) saw populations of lactic acid bacteria rise 4.7% in the cecum of the horse within 24 hours of switching diets from all-forage diet to an all-concentrate diet. The increase in these types of bacteria can result in an accumulation of lactic acid in the cecum which may lead to changes to the hindgut environment which are believed to be linked to laminitis.

The proliferation of lactic acid bacteria have been implicated as one of the major causes of acute laminitis in the horse, a condition in which the sensitive laminae that connects the coffin bone to the hoof wall is broken down. Lamintis is associated with lactic acidosis, which can be induced by the over production of lactate in the cecum of the horse when they consume large amounts of concentrate feeds containing starch. Garner et al. (1978) found that by administering 17.6g/kg BW of cornstarch-wood flour gruel via stomach tube, that laminitis can be induced in horses. After this induction, *Lactobacillus* spp. drastically increased within 8 hr, while *Streptococcus* spp. decreased. An *in vitro* model of carbohydrate overload found that populations of *Streptococci* spp.

and *Lactobacilli* spp. increased within 12 hr of starch being added to samples (Bailey et al., 2003). Exactly how the increase in bacteria triggers laminitis in horses has yet to be determined. However, Bailey et al. (2002 and 2003) suggest that the production of vasoactive amines, which were observed to increase during carbohydrate overload, may play a role in causing laminitis in horses. The increase in lactic acid caused by these bacteria has also been linked to an increase in the population of lactic acid-utilizing bacteria in the equine cecum.

Lactate-utilizing bacteria were first detected in the equine colon by Alexander et al. (1952), and have since been a population of interest when lactic acid bacteria concentrations increase in the cecum. When Goodson et al. (1988) abruptly changed diets from forage to concentrate in horses, the population of lactate-utilizing bacteria increased after 3 to 7 d, representing up to 69.2% of the total bacterial numbers. The population then decreased and stabilized after 2 wk at 33.5% (Goodson et al., 1988). The gradual adaptation to high grain diets also caused increases in lactate-utilizing bacteria in the cecum of horses, increasing from 2.45×10^5 /mL when an all-hay diet was fed to 1.50×10^6 /mL when the a 50% hay-50% barley diet was fed (Julliand et al., 2001). However, too much starch can be detrimental to these bacterial populations. When Garner et al. (1978) induced alimentary laminitis the populations of lactate-utilizing bacteria decreased after 8 hr. This decrease is believed to be caused by these organisms inability to tolerate acidic environments below pH 6.2 (Leek et al., 1977). A similar reaction has been noted for other cecal microorganisms, such as cellulolytic bacteria.

The increase in amylolytic bacteria is often followed by a decrease in acid intolerant species such as cellulolytic bacteria. This has been demonstrated numerous times in ruminant species when diets have been changed both abruptly and gradually (Hungate et al., 1952; Bryant and Burkey, 1953; Grubb and Dehority, 1975; Leedle et al., 1982). Kern et al. (1973) did not notice any effect on cellulolytic bacteria when oats were added to forage only diets, but this may be attributed to the low level of grain added to the diet (25% of the diet). Further studies in horses have observed decreases in cellulolytic bacteria similar to ruminants when the small intestine was overloaded with starch (2 - 4 g/kg BW), resulting in changes to bacterial populations in the large intestine (Potter et al., 1992; Meyer et al., 1995.; Julliand et al., 2001; Medina et al., 2002). When alimentary laminitis was induced in horses, Gram-negative bacteria, which are typically cellulolytic, decreased within 8 hr (Garner et al., 1978). While the changes to cellulolytic bacteria have been predictable, the protozoa concentrations in the cecum have had more varied results.

The information on how protozoa respond to diet is much more limited than that of cellulolytic and amylolytic bacterial species. Research in sheep has found that protozoa populations increase when concentrate is added to hay-only diets from 5.3 x 10^5 to 9.5 x 10^5 protozoa/cm^3 of rumen contents (Grubb and Dehority, 1975). When horses were adapted to diets comprised of concentrates, no significant change was observed to the population of protozoa in the cecum (Kern et al., 1973; Moore and Dehority, 1993). Abrupt incorporation of concentrate, however, elicited a decrease in the protozoan population, opposite of the response recorded in the rumen of sheep

(Grubb and Dehority, 1975; Goodson et al., 1988). The scant information on the purpose of protozoa in the horse could lead insight as to this variation in results.

Changes to Fermentation Products. The products of microbial fermentation are important in providing for the nutrient needs of the horse. When bacterial populations change in response to diet, the products of fermentation available to the horse also change. Ruminants fed increasing levels of concentrate have noticeably higher concentrations of VFAs as a result of the increased total bacteria in the rumen (Rumsey et al., 1970). When horses were allowed to adapt to a diet including concentrate, no significant change in total VFAs were observed by researchers when compared to hay-only diets (Kern et al., 1973; Medina et al., 2002). However, the abrupt change from a 100% hay diet to a 50% hay-50% barley diet did cause a significant increase in the total VFA concentrations (de Fombelle et al., 2001). The noted increase may be due to the sudden proliferation of bacteria; while in the studies by Kern et al. (1973) and Medina et al. (2002) bacterial populations were allowed to stabilize.

The increase in total VFAs may be attributed to a rise in lactic acid which has been observed in both ruminants and horses when concentrate is introduced to the diet (Latham et al., 1971; Garner et al., 1975; Medina et al., 2002). Medina et al. (2002) saw lactic acid in the cecum of the horse increase when horses were fed either a high fiber diet or a high starch diet (3.4 g starch/kg BW/meal). This reaction was also observed by Al Jassim et al. (2005) and Respondek et al. (2008) and was attributed to the proliferation of amylolytic bacteria. The lactic acid produced by these bacteria can be converted into propionate through the acrylate pathway, resulting in an increase in

propionate concentrations when NSCs are incorporated into the diet (Hungate, 1966; Hintz et al., 1971). When Hintz et al. (1971) increased forage-grain ratios (1:0 to 1:4), the molar percentage of propionate increased from 14.8 to 26.0. While the propionate and lactate increase with increased dietary starch, the cecal environment becomes more acidic, resulting in a decrease in cellulolytic bacteria and an accompanying decrease in the production of acetate. The optimal pH for cellulolytic bacteria is above 6.2, and when the cecal pH drops below this level the production of acetate diminishes (Leek et al., 1977). These changes result in a decrease in the acetate to propionate ratio in the cecum (Hintz et al., 1971; deFombelle et al., 2001). The concentration of butyrate has seen little change with respect to increasing amounts of NSCs in the diet. Hintz et al. (1971) observed an increase in the molar percentage of butyrate from 8.7 to 12.5, suggesting that butyrate-producing bacteria also benefit from an increase in NSCs. While other research has noticed a slight increase in butyrate concentrations in similar conditions, Hintz et al. (1971) is the only study to have a significant increase (Kern et al., 1974; de Fombelle et al., 2001; Medina et al., 2002; Respondek et al., 2008). Although VFAs are the primary focus of most research due to their importance as energy substrates, changes also occur to methane and ammonia when diets include NSCs.

The changes to the microflora also result in alterations to other products of fermentation such as ammonia and methane. The decrease in pH caused by the accumulation of lactic acid has a negative effect on the production of methane. Wolin (1960) suggested than an inverse relationship existed between propionate and methane concentrations, with current research lending validity to this, noting the ability of

bacteria to produce methane is highly influenced by the ratio of acetate to propionate (Lana et al., 1998). Bacteria dispose of reducing equivalents through either methanogenesis or propionate production. When NSCs are incorporated into the diet the amount of lactate increases and production of propionate occurs rather than methanogenesis resulting in a decrease in methane. This has been observed in both ruminants and horses (Hirose et al., 1957; McDaniel et al., 1993; Lana et al., 1998). In vitro fermentation of cecal fluid with bermudagrass hay and soluble starch resulted in methane production decreasing from 1.49 mmols to 0.26 mmols (McDaniel et al., 1993). When diets include NSCs, the amount of available ammonia in the cecum also decreases.

Ammonia concentrations have a tendency to decrease as concentrate is added to the diets of both ruminants and horses (McDaniel et al., 1993; Lana et al., 1998). The in vitro fermentation of starch produced 8.8 mg/L of ammonia, while bermudagrass hay produced 373.7 mg/L when using equine cecal fluid (McDaniel et al., 1993). This decrease in ammonia has been linked to several possible factors such as increased microbial protein synthesis and decreased deamination rates (Lana et al., 1988). The increase in microbial protein synthesis would cause ammonia to decrease by assimilating these molecules into amino acids, while less deamination would mean fewer amino acids are broken down, releasing less ammonia into the cecal environment. The decrease in deamination rate is believed to be caused by the populations of cellulolytic bacteria decreasing in response to the lower pH that often follows high concentrate meals.

Changes in Cecal pH. The addition of dietary NSCs increases the population of amylolytic bacteria, and thereby increases the production of VFAs lactate and propionate. These organic acids can overwhelm the buffering capacity of the host's digestive tract, resulting in a decrease in pH. Lactic acid has been targeted as a major cause for the decrease in pH, due to its lower pK value (3.9) compared to other VFAs such as acetate which has a pK value of 4.8 (Frape, 2004). At a pH value of 6.2 the cecal environment is no longer considered optimal for lactic acid-utilizing bacteria, deterring the conversion of lactic acid into propionate, driving the decline in pH further (Leek et al., 1977). This allows the amylolytic bacteria to predominate in the environment, adding to the production of lactic acid. This decrease can be observed between 4 – 6 hrs after feeding; at about the time the majority of digesta reaches the cecum (Argenzio et al., 1974a; Willard et al., 1977). When Willard et al. (1977) fed concentrate to horses, pH in the cecum decreased from 7.22 at 0 hrs to 6.12 at 6 hrs. The pH of the horses fed hay only dropped to 6.87 at 6 hrs from a starting pH of 7.14 (Willard et al., 1977). The decrease is dependent on the amount of concentrate in the diet, as well as the tendencies of the individual horse. When alimentary laminitis was induced in horses the pH in the cecum plummeted from 7.18 to 5.72 within 8 hrs, and further dropped to 4.14 over 24 hrs (Garner et al., 1978). If the pH drops below 6.0, the horse is considered to have subclinical acidosis cecal acidosis, and may eventually have damage to the mucosal barrier (Clarke et al., 1990; Radicke et al., 1991). In order to prevent these types of digestive upsets, more research is required to determine the role of dietary NSC concentrations relative to the cecal environment.

Conclusions

The research performed in ruminants can give important clues in how the cecum of the horse will respond when exposed to concentrate meals. The pre-cecal physiology of the horse, however, prevents equine researchers from being able to directly apply data found in ruminants to the horse's digestive tract, although trends can be observed. While having some starch reach the cecum is not unhealthy, the over abundance of it may eventually lead to acidosis or laminitis in the horse. With the horse industry's dependence on concentrate feeds, it is important to know exactly where that detrimental line is. By knowing how much starch is required to elicit specific changes in cecal environment it could allow for the better formulation of diets for performance horses without worry of possible digestive problems.

CHAPTER II

MATERIALS AND METHODS

Horses and Dietary Treatments

Eight mature geldings (Quarter horse cross) were obtained from the Texas Department of Corrections for use in this experiment. Horses ranged in age from 5 to 12 years and had an average BW of 585 kg (± 50.11 kg). Between June and November of 2007 horses were cecally fistulated and equipped with a 3.5 cm cannula (Bar Diamond, Inc. Parma, IN). The first 2 horses were fistulated using a procedure similar to that of Wilkins and Lowe (1993), while the remaining horses (n = 6) underwent a standing surgery. The horses were allowed at least one month recovery time in individual stalls before being group housed in 12 x 14.5 meter dry lot pens. Prior to the start of the study, horses underwent a backgrounding period for a minimum of 1 month, with horses being fed pelleted concentrate feed (13% CP pellet, Producers Cooperative, Bryan, TX) and bermudagrass hay (*Cynodon dactylon*) to allow for uniformity of cecal environment.

Horses were paired and randomly assigned to dietary treatments within a replicated 4 x 4 Latin square design (Table 1), with each treatment period lasting 21 days. The 4 dietary treatments contained increasing concentrations of starch: 2 g starch/kg BW/d (Diet 2), 4 g/kg BW/d (Diet 4), 6 g/kg BW/d (Diet 6), 8 g/kg BW/d (Diet 8). The starch concentration of each diet was met through a combination of a commercial pelleted feed (SafeChoice™, Nutrena) and corn meal. Diets were isonitrogenous through the addition of soybean meal, and DMI was constant among diets by the addition of cottonseed hulls to the rations. The nutrient profile of these ration

components was analyzed by a commercial laboratory (SDK Laboratories, Hutchinson, KS) prior to the start of the study, and the dietary nutrient composition was calculated from these results (Table 2). All diets were formulated on a % BW basis with bodyweights of the horses measured on d 0 of each treatment period to insure accuracy. The composition of the concentrate diets can be found in Table 3.

Table 1. Latin square experimental design designating the treatment diet of the horses for each period.

Horse Number	Diet[1]			
	Period 1	Period 2	Period 3	Period 4
1, 2	2	6	8	4
3, 4	4	2	6	8
5, 6	8	4	2	6
7,8	6	8	4	2

[1]Diets: 2 = 2g starch/kg BW/d; 4 = 4g starch/kg BW/d; 6 = 6g starch/kg BW/d; 8 = 8g starch/kg BW/d.

Table 2. Nutrient analysis of ration components and calculated treatment diets (%, DM basis).

	Ration Components[1]					Diets (calculated)[2]			
Item	Pellets	CM	SBM	CSH	BGH	2	4	6	8
ADF	19.64	3.98	7.62	64.72	36.58	69.52	54.63	39.74	24.86
NDF	33.81	10.00	10.62	80.73	69.36	97.10	79.90	62.69	45.50
CP	15.30	6.60	49.70	5.67	7.36	26.35	25.02	23.66	22.34
Starch	20.40	81.40	3.30	1.90	3.70	24.14	46.27	68.48	90.69
Ca	1.46	0.03	0.56	0.16	0.37	1.78	1.73	1.64	1.63
P	0.75	0.24	0.77	0.13	0.11	1.00	1.01	1.02	1.02

[1] Components: CM = Corn Meal; SBM = Soybean Meal; CSH = Cottonseed Hulls; BGH = Bermudagrass Hay.
[2] Diets: 2 = 2g starch/kg BW/d; 4 = 4g starch/kg BW/d; 6 = 6g starch/kg BW/d; 8 = 8g starch/kg BW/d. Hay was fed separately to the horses at 0.5% BW.

Table 3. Ration formulations for each dietary treatment (as % BW AF).

	Ration Components				
Diet[1]	Pellets	Corn Meal	Soybean Meal	Cottonseed Hulls	Total Intake
2	1.10	--	0.11	0.73	1.94
4	1.10	0.28	0.07	0.49	1.94
6	1.10	0.56	0.04	0.24	1.94
8	1.10	0.84	--	--	1.94

[1] Diets: 2 = 2g starch/kg BW/d; 4 = 4g starch/kg BW/d; 6 = 6g starch/kg BW/d; 8 = 8g starch/kg BW/d.

Horses were fed twice daily, at 0600 and 1600 in individual stalls (3.05 x 3.05 m) and allowed 2 hr to consume the concentrate diet. Grain refusals were weighed and recorded. Hay was offered at 0.5% BW in dry lot pens (12.0 x 14.5 m) to each pair of horses. The nutrient analysis for the bermudagrass hay can be found in Table 2. Horses

were housed in dry lot pens and allowed free exercise and ad libitum access to water. During the first 6 days of each treatment period horses were gradually adapted to dietary treatments and received the entire amount beginning on day 7.

Sample Collection

Cecal digestive contents were collected on day 21 of each treatment period. The morning of each sample collection day the horses were fed in a staggered feeding schedule (n = 4) with one horse from each treatment pair fed at either 0600 or 0630. This allowed for a smaller number of horses to be collected at one time. Cecal samples were taken at approximately 4 h after the consumption of the meal, when cecal pH begins to decline (Willard et al., 1977). Cannulas were opened and cecal content (liquid and solid) were collected in 350 mL insulated containers. The pH of the cecal contents was immediately analyzed using a handheld pH meter (Thermo Orion, West Chester, PA). Approximately 20 mL of cecal fluid was strained through two layers of cheesecloth into 50 mL conical vials and frozen at -20^{o}C for later analysis of VFA. Once all cecal samples were collected, the insulated containers were transported to the Food & Feed Safety Unit of the Southern Plains Agricultural Research Unit, USDA/ARS facility for further analysis. Blood samples were also obtained at this time via jugular venipuncture using tubes containing sodium heparin (BD Diagnostics, Franklin Lakes, NJ). The blood samples were centrifuged at 2700 g for 20 min and then harvested for plasma and frozen at -20^{o}C for later analysis.

Microbial Analyses

Upon arrival at the Bovine Microbiology Laboratory at Southern Plains Agricultural Research Unit the samples were placed in a Bactron Anaerobic/Environmental Chamber (Sheldon Manufacturing, Inc. Cornelius, OR) with a 90% N_2 – 5% CO_2 – 5% H_2 atmosphere. Samples were prepared in a series of 10-fold serial dilutions in an anaerobic mineral solution (Bryant and Burkey, 1953) from 10^{-1} to 10^{-9} to be inoculated on specific media for bacteria enumeration. Anaerobic Brucella Blood (BRU) agar plates (Anaerobe Systems, Morgan Hill, CA) were inoculated with the serial dilutions (0.1 mL/plate) in the Bactron Chamber to enumerate total culturable anaerobes (Mangels and Douglas, 1989). Plates were incubated at 37°C for 72 hr before bacterial colonies were counted and recorded. The number of lactic acid bacteria was determined using two different types of selective media: DeMan Rogosa Sharpe (MRS) agar (Ghorbani et al., 2002) and Rogosa agar (Julliand et al., 2001). Difco™ Rogosa SL agar (Becton Dickson and Company, Sparks, MD) was prepared in petri dishes, and placed in the Bactron Chamber prior to sample collection. The medium was inoculated and spread with the serial dilutions at 0.1 mL/plate in the Bactron Anaerobic Chamber and were incubated for 48 hr at 37°C before bacteria were counted and recorded. Difco™ Lactobacilli MRS Agar was also prepared in petri dishes prior to sample collection, and then inoculated aerobically with the serial dilutions (0.1 mL/plate); the plates were then incubated in a Steri-Cult CO_2 Incubator (Forma-Scientific, Mariett, OH) with a 5% CO_2 atmosphere at 35°C for 72 hr before bacterial counts were conducted. A

40 mL sample of cecal fluid was stored in a 50 mL tube and stored at -20°C until laboratory analyses.

Methane and Ammonia Activity

Methane-producing activity was determined through an in vitro incubation of cecal contents, using a procedure by Anderson et al. (2006). The procedure required 2 g of cecal contents, 8 mL of anaerobic dilution solution (Bryant and Burkey, 1953) containing 60 mM sodium formate, and 0.2 g of ground alfalfa to be mixed in 18 x 150 Hungate culture tubes under an H2:CO2 (50:50) gas phase, with each sample performed in triplicate. The tubes were capped and incubated for 3 hr at 39°C before headspace of each tube was analyzed for composition using a gas chromatograph (Gow-Mac Instrument Co., Bethlehem, PA). The headspace gas in each tube was removed once the analysis was completed; the tubes were then placed in a 39°C incubator for 48 hr to allow for further in vitro fermentation. Tubes were then stored at -20°C for later determination of in vitro dry matter digestibility.

Ammonia-producing activity was determined using the colorimetric assay of Chaney and Marbach (1962).The assay used a catalyzed indophenol reaction to determine ammonia content by producing a blue color. The samples were then read by a SpectraMax 340 PC spectrophotometer (Molecular Devices, Sunnyvale, CA) and the data was acquired and the absorbance of each sample was compared to known standards using SoftMax Pro software (Molecular Devices, Sunnyvale, CA).

VFA Analyses

Cecal samples were acidified using metaphosphoric acid (5:1 ratio sample:acid), frozen, and shipped to Kansas State University for VFA analysis. The VFA concentrations were determined by gas chromatograph (Model 5890, Hewlett Packard, Avondale, PA) with a flame ionization detector, using the procedure described by Vanzant and Cochran (1994). The chromatograph was fitted with a 1.8-m, 4-mm id glass column, packed with 10% SP1200/1% H_3PO_4 on 80/100 WAW Chromsorb (Supleco, Bellefonte, PA). The column was maintained at 140°C, while the detector and injector remained at 225°C. The samples were injected into the GC, volatilized, and transported by a N_2 carrier gas at a flow of 80 mL/min into separation column.

Stoichiometric Calculations

The approximate amount of hexose fermented was estimated using VFA concentrations according to the equation developed by Chalupa (1977):

Hexose fermented = ½ acetate + ½ propionate + butyrate + valerate

The approximate amount of CO_2 and CH_4 produced in the cecum was estimated using the results of the VFA analysis in a series of fermentation balance equations described by Wolin (1960).

In Vitro Dry Matter Digestibility

The DM percentage of the cecal contents was determined by drying 1 g of cecal contents in a 105°C oven for 14 hr. An in vitro dry matter digestibility (IVDMD) was performed using the samples that were previously prepared for methane analysis. The assay used a modification of the method described by Tilley and Terry (1963) to

determine the IVDMD of 0.2 g of ground alfalfa and 2 g of cecal contents. Each 18 x 150 Hungate tube contained 2 g of cecal contents, 8 mL of anaerobic dilution solution with 60 mM sodium formate and 0.2 g of ground alfalfa. These contents were filtered through pre-weighed filter paper (Whatman no. 541) under a low vacuum and rinsed with 15 mL of double distilled water to remove any soluble material, leaving only the undigested portion. The filter paper with sample was then dried at 105°C for 8 hr and then weighed to find a DM end weight for the sample. The DM start weight of the sample was then calculated by adding the DM of the sample and the DM of the alfalfa. The following equation was then used to determine the DM digestibility of the sample:

$$\text{Digestibility} = \frac{\text{DM start weight} - \text{DM end weight}}{\text{DM end weight}}$$

Statistical Analyses

Data were analyzed by ANOVA using the PROC GLM procedure of SAS (SAS Inst. Inc., Cary, NC) with diet, period, and horse in the model statement. Data from all animals were included in the analysis. Logarithmic transformations were preformed on bacterial counts before statistical calculations were conducted. Linear, quadratic, and cubic effects were tested in the form of contrasts. In all cases, an alpha level of 0.05 was used for determination of statistical significance. Probability values between 0.05 and 0.10 were deemed to be trends toward significance.

CHAPTER III

RESULTS AND DISCUSSION

Dry Matter and Starch Intake

Daily dry matter intake (DMI) of concentrate did not differ ($P < 0.70$) with dietary treatment. However, dry matter intake of concentrate tended to be influenced by period ($P = 0.08$) and horse ($P < 0.06$). The horses consumed the greatest percentage of Diet 6, 49%, and the least of Diet 8 at 38%. Diets 2 and 4 were also within this range at 46% and 42%, respectively. Even though the horses in this study did not consistently consume all of their treatment diets, there were few instances of sorting observed, allowing daily starch intake to be calculated. As expected, daily starch intake was affected by treatment ($P < 0.0001$), increasing linearly ($P < 0.0001$) as the concentration of starch offered in the diet increased (Table 4). Individual horse also had an effect ($P < 0.04$) on the amount of starch consumed in this study which can be related to the differences in DMI observed in individual horses. According to previous research by Potter et al. (1992) and Meyer et al. (1995) the amount of starch required to overwhelm the capacity of the small intestine is between 2 – 4 g/kg BW/meal. Due to the inconsistent intake of the horses, starch intake did not exceed 3.19 g/kg BW/d or 1.60 g/kg BW/meal, which is below the range described by Potter et al. (1992) and Meyer et al. (1995). Because of this, there may not have been sufficient amounts of starch to reach the cecum of the horses in this study.

Table 4. Dry matter and starch intake of horses fed graded levels of dietary starch.

	Diet[1]			
Item	2	4	6	8
Total Offered	11.18	11.06	11.12	11.24
Dry Matter Intake, kg/d	5.16[a]	4.64[a]	5.48[a]	4.35[a]
Starch Offered, kg/d	1.15	2.28	3.44	4.65
Starch Intake, kg/d	0.53[a]	0.96[a]	1.71[b]	1.82[b]
Starch Intake, g/kg BW/d	0.91	1.66	2.99	3.19

[1] Diet: 2g starch/kg BW/d; 4g starch/kg BW/d; 6g starch/kg BW/d; 8g starch/kg BW/d.
[a-c] Means within a row with different superscripts differ ($P < 0.05$).

Cecal pH

Cecal pH tended to decrease linearly ($P < 0.06$) as the amount of starch in the diet increased (Figure 1). Diet 6 elicited the lowest pH (6.5) which can be contributed to the greater DMI of this diet in comparison to the other treatment diets. Due to the low DMI of Diet 8, the starch intake of Diet 6 and 8 was relatively similar. This is reflected in the cecal pH values of 6.51 and 6.58 for Diets 6 and 8, respectively. Diet 6 elicited a lower pH value than Diet 2 ($P < 0.04$), which can be related to the higher starch and DM intake in Diet 6. There was also a tendency towards an influence of period ($P < 0.07$) on cecal pH, which could also be related to the DMI differences depending on treatment period. The pH range in this study was similar to those found by Kern et al. (1973) when horses were fed hay with and without the addition of oats. The lowest average pH, found in Diet 6, was comparable to horses fed a diet of clover hay and oats in the Kern et al. (1973) study (approximately 1 g starch/kg BW/meal). The linear decrease in cecal pH as the amount of starch in the diet increased is similar to data in previous studies in the horse (Garner et al., 1978; deFombelle et al., 2001; Julliand et al., 2001; Medina et

al., 2002) and in ruminants (Hungate et al., 1952; Leedle et al., 1982; Lana et al., 1998). This decrease is often attributed to an accumulation of VFAs, specifically lactate, in the cecum or rumen. The average cecal pH, regardless of treatment diet, remained above 6.0, which is considered by Radicke et al. (1991) to be the threshold for subclinical lactic acidosis. Over the course of the study, only one horse recorded a pH below this range, at 5.63, although no symptoms of subclinical acidosis were observed.

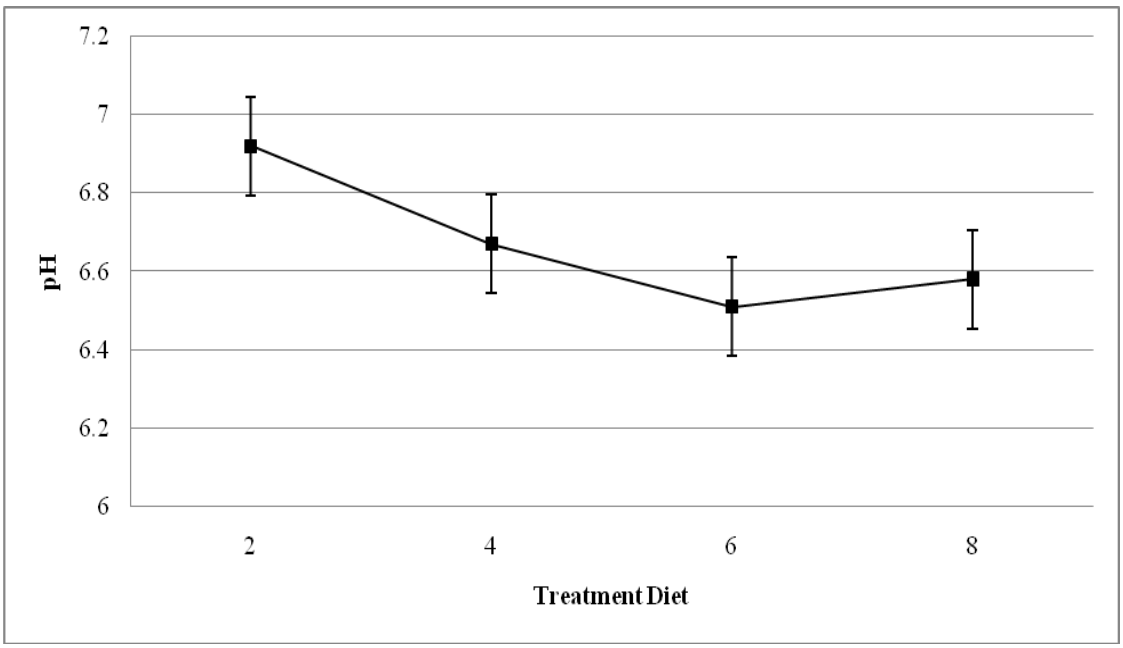

Figure 1. The effect of graded levels of starch on the pH in the cecum of the horse. Diet 2 = 2g starch/kg BW/d, Diet 4 = 4 g starch/kg BW/d, Diet 6 = 6g starch/kg BW/d, 8 = 8g starch/kg BW/d.

Microbial Populations

There was no effect ($P > 0.10$) of dietary treatment on populations of total culturable anaerobes (Table 5). This lack of response may be due the low levels of starch intake, not allowing sufficient amounts of starch to reach bacteria in the cecum.

Previous studies have correlated an increase of starch in the diet with an increase in the total anaerobic bacteria in the cecum (Kern et al., 1973; Julliand et al. 2001; Medina et al., 2002). The average total anaerobic bacteria in the current study ranged from 5.83×10^8 to 1.58×10^9 c.f.u./mL, which falls within the range observed in previous studies. Kern et al. (1973) noted slightly larger populations of total anaerobes in hay diets with and without oats, counting 4.58×10^9 to 7.02×10^9 per g ingesta. While Medina et al. (2002) saw an increase in total anaerobic bacteria from 7.9×10^7 to 4.0×10^8 c.f.u./mL. These variations may be attributed to the use of different starch sources. Kern et al. (1973) used oats while Medina et al. (2002) used barley, which contains a less degradable form of starch (Meyer et al., 1995).

There was no influence of dietary treatment on lactic acid bacteria populations ($P > 0.10$). Lactic acid bacteria ranged from 1.10×10^7 to 1.00×10^8 c.f.u./mL on the MRS agar and 4.4×10^7 to 1.55×10^8 c.f.u./mL on the Rogosa agar (Table 5). There was no previous data reported using MRS agar for equine cecal fluid. Medina et al. (2002) used Rogosa agar and found similar populations of lactic acid bacteria, counting 5.01×10^7 c.f.u./mL of cecal contents when horses were fed a high starch diet. Julliand et al. (2001) also used Rogosa agar to detect *Lactobacillus* spp. populations in horses fed different hay:grain ratios (not exceeding 2.3 g starch/kg BW/meal), finding 4.17×10^5 – 7.34×10^6 c.f.u./mL. While Julliand et al. (2001) observed lower populations, their study utilized a smaller number of horses, allowing a greater influence of individual variation on that data.

The lack of population increase in the current study could be due to the more subtle decrease in cecal pH recorded in this experiment. The production of lactic acid is a major proponent in the decrease in pH, and the acidic environment benefits these bacteria, allowing the populations to proliferate (Kern et al., 1973; Garner et al., 1978; Frape 1997; Julliand et al., 2001). However, because starch intake was lower, it would appear that the bacteria did not produce enough lactic acid to make an acidic environment that would benefit predominately the lactic acid bacteria in the cecum. Furthermore, because the populations did not consistently increase, the total culturable anaerobes did not reflect the steady increase that is associated with high starch diets.

Table 5. Microbial populations in the cecum of horses fed graded levels of dietary starch.

	Diet[1]				
Item, log_{10} c.f.u./mL	2	4	6	8	SEM
Total Culturable Anaerobes	8.94[a]	9.20[a]	8.71[a]	8.84[a]	0.30
Lactic Acid Bacteria					
MRS	7.48[a]	8.00[a]	7.04[a]	7.28[a]	0.35
Rogosa	7.73[a]	7.65[a]	7.75[a]	8.19[a]	0.32

[1] Diet: 2 = 2g starch/kg BW/d; 4 = 4g starch/kg BW/d; 6 = 6g starch/kg BW/d; 8 = 8g starch/kg BW/d.
[a-c] Means within a row with different superscripts differ ($P < 0.05$).

Cecal Ammonia and Methane

Dietary treatment had no effect ($P > 0.10$) on the concentration of ammonia in the cecum (Table 6). The minimal changes observed with bacterial populations could be related to the few changes seen in ammonia production. The ammonia concentrations

found in the current study were similar to those reported by McDaniel et al. (1993) through in vitro fermentation of cecal fluid with bermudagrass hay (373.7 mg/L) or alfalfa hay (730.0 mg/L). Although the diet did not significantly affect ammonia production in the cecum, there were numerically lower ammonia concentrations when higher starch diets were consumed. This coincides with data from Lana et al. (1998) which found that high starch diets caused a decrease in ruminal ammonia concentrations in cattle. The data collected for methane production will not be included due to inconsistencies with the gas chromatograph over the course of the experiment.

Volatile Fatty Acids

Dietary treatment had no effect ($P > 0.10$) on the production of acetate. However, there was a tendency towards a cubic effect ($P < 0.07$) of dietary starch on cecal acetate concentrations, with increasing concentrations of acetate with Diets 2 – 6, then a decline with Diet 8 (Table 6). There was also an influence of period ($P < 0.05$) on cecal acetate production. These concentrations of cecal acetate are similar to the 34.6 mM of acetate found in the cecum of horses fed a diet of Timothy hay and oats by Kern et al. (1973). These values are lower, however, than those recorded by Medina et al. (2002) which observed acetate concentrations of 50.9 and 43.4 mM when horses were fed a high fiber or high starch diet. The reason for the higher acetate concentration in the Medina et al. (2002) study could be contributed to the greater DMI (21 g/kg BW/d) than the current study (7.42 – 9.37 g/kg BW/d) allowing more substrate for the bacteria to ferment. According to Leek et al. (1977) the optimal pH for cellulolytic bacteria is above 6.2, and if pH drops below this then acetate production will begin to decline.

However, only one horse in the current study recorded a pH below this threshold, signifying that the cecal environment in most cases was still sufficient for cellulolytic bacteria.

Cecal propionate production was also influenced ($P < 0.04$) by dietary treatment. A quadratic effect was observed in this experiment ($P < 0.05$) as well as a tendency for a cubic effect ($P < 0.06$). The highest propionate production was observed in Diet 6, with the lowest in Diet 2, which can be attributed to the differences in starch intake (Table 6). This increase in propionate production is consistent with previous research that observed higher cecal propionate concentrations when diets including starch were compared to forage diets (Hintz et al., 1971; Kern et al., 1973; Medina et al., 2002). This increase illustrates that some dietary starch was able to reach the amylolytic bacteria in the cecum, even with the lower starch intake that occurred in the current study. Hintz et al. (1971) observed an increase in propionate from 8.48 – 12.43 mM when horses were fed increasing forage:grain ratios (approximately 2.15 – 3.71 g starch/kg BW/meal), which is similar to the range observed in the current study. Kern et al. (1973) and Medina et al. (2002) also noted similar concentrates of propionate when comparing forage only diets to diets where starch was added. Kern et al. (1973) observed propionate increases from 10.7 – 17.5 mM, while propionate concentrations in the Medina et al. (2002) study increased from 12.8 – 17.7 mM.

The treatment diets had a tendency ($P < 0.08$) to cause changes to the production of butyrate in the cecum of the horses in this experiment. Additionally, butyrate concentrations tended to have a quadratic response ($P < 0.09$) when the level of starch in

the diet increased, peaking when the horses were fed Diet 6 (Table 6). This trend in butyrate production coincides with previous research that noted increased butyrate production when starch was added to the diet of horses (Hintz et al., 1971; Kern et al., 1973; Medina et al., 2002). The concentrations found in the current study, however, are slightly lower than those noted in previous research. When Hintz et al. (1971) increased hay:grain ratios, butyrate increased from 3.57 mM on the hay only diet to 4.65 mM on the high grain diet (2.85 g starch/kg BW/meal). Kern et al. (1973) and Medina et al. (2002) found butyrate concentrations ranging from 3.3 – 5.4 mM when comparing forage diets to starch added diets. The reason for the lower butyrate concentrations in the present study is unknown.

There were no changes to the production of isobutyrate ($P < 1.00$), isovalerate ($P < 1.00$), and valerate ($P < 0.60$) in response to the different levels of dietary starch (Table 6). The concentration of isobutyrate in the current study is similar to that of Hintz et al. (1971), which noted values from 0.22 – 0.34 mM when the hay:grain ratios were altered. The current study also observed isovalerate and valerate concentrations which fell within the range described by Hintz et al. (1971) of 0.11 – 0.43 and 0.29 – 0.57, respectively. The concentration of isobutyrate and isovalerate in the current study was lower in the two higher starch diets (Diets 6 and 8) than that of the lower starch diets (Diets 2 and 4), differing from the data noted by Hintz et al. (1971) that saw an increase in these VFAs as the amount of grain in the diet increased. The reason for this discrepancy can be attributed to the lower starch intake of the current study, since starch intake did not

exceed 1.60 g/kg BW/meal while the horses in the Hintz study were fed up to approximately 2.85 g/kg BW/meal.

The acetate:propionate ratio in this study ranged from 2.6 (Diet 6) to 3.31 (Diet 2), while Diets 4 and 8 recorded values of 3.17 and 2.90, respectively. This coincides with previous research which noted a decrease in the acetate:propionate ratio with the inclusion of starch into the diet of horses (Hintz et al., 1971; Kern et al., 1973; Medina et al., 2002). Although both acetate and propionate production increased with greater amounts of dietary starch, the amylolytic bacteria in the cecum benefited more, resulting in a higher proportion of propionate. Hintz et al. (1971) found similar acetate:propionate ratios, with values ranging from 2.38 to 3.85 when horses were fed differing hay:grain ratios. The current study also fell within the range described by Medina et al. (2002), which had an acetate:propionate ratio of 3.97 and 2.45 when horses were fed either a high fiber or high starch diet.

Table 6. Volatile Fatty Acid analysis and ammonia production in the cecum of horses fed graded levels of dietary starch.

	Diet[1]				
Item[2]	2	4	6	8	SEM
Volatile Fatty Acids, mM					
Acetate	27.39[a]	30.31[a]	36.86[a]	27.05[a]	3.27
Propionate	8.26[a]	9.55[a]	14.13[b]	9.25[a]	1.40
Butyrate	2.05[a]	2.35[a,b]	3.16[b]	2.38[a,b]	0.29
Isobutyrate	0.32[a]	0.37[a]	0.29[a]	0.27[a]	0.13
Isovalerate	0.28[a]	0.36[a]	0.25[a]	0.23[a]	0.14
Valerate	0.28[a]	0.35[a]	0.37[a]	0.27[a]	0.06
Ammonia, mg/mL	0.58[a]	0.76[a]	0.38[a]	0.46[a]	0.37

[1] Diet: 2 = 2g starch/kg BW/d; 4 = 4g starch/kg BW/d; 6 = 6g starch/kg BW/d; 8 = 8g starch/kg BW/d.
[2] Each mean represents 7 individually fed horses.
[a-b] Means within a row with different superscripts differ ($P < 0.05$).

Stoichiometric Calculations

According to stoichiometric calculations, the amount of hexose fermented in the cecum had a tendency ($P < 0.09$) to be effected by the varying levels of starch in the treatment diets. A tendency for a quadratic effect ($P < 0.06$) was noted, with Diet 6 providing the highest amount of fermented hexose which could be related to the greater DMI and consequently greater starch intake of this treatment. There was also a tendency ($P < 0.06$) for the treatment period to effect hexose fermentation, which is consistent with the period effect noted in for DMI. The increase in fermented hexose indicates that increasing amounts of readily fermentable starch were made available to the cecal bacteria over the course of the experiment. A search of the literature found no previous use of stoichiometric calculations to predict fermentation in horses. Because of this, the use of stoichiometric calculations can only be used as indicator of microbial activity rather than a direct analysis.

There was no response to diet noted in the production of CO_2 ($P < 0.20$) in this experiment. However, a tendency towards a quadratic effect ($P < 0.06$), with Diet 6 producing the greatest amount of CO_2 was observed (Table 7). Period also had a tendency to effect CO_2 production ($P < 0.06$) which could be the result of difference in intake over the treatment periods. There was no previous research observing the concentration of CO_2 in the cecum of horses, however, according to Beuvink and Spoelstra (1992) when glucose is fermented into acetate, CO_2 is released both as a by-product of fermentation and from the buffer when the acetate is produced. Fermentation producing propionate only releases CO_2 through the buffer, showing that in the case of the current experiment, the increasing concentration of acetate and propionate results in an increase of CO_2 in the cecum.

Methane production was not affected by the varying levels of starch in the treatment diets ($P > 0.10$). However, the data had a tendency to exhibit a quadratic effect ($P < 0.09$) when the starch concentration of the diets increased (Figure 3). Methane production differed significantly ($P < 0.04$) depending on the treatment period, which coincides with the effect period had on DMI. The increase observed in methane production is the opposite of what was described by Wolin (1960). According to Wolin (1960) and Lana et al. (1998) as propionate increases and acetate decreases, the tendency of bacteria to undergo methanogenesis decreases. However, because the pH in the cecum did not decrease enough to discourage cellulolytic populations, it is possible that the bacteria were still able to participate in methanogenesis as well as propionate production, resulting in the apparent increase in methane in this study.

Table 7. Stoichiometric calculations of fermented hexose, methane, and carbon dioxide for horses fed graded levels of dietary starch.

	Diet[1]				
Item, mM	2	4	6	8	SEM
Hexose fermented	20.15[a]	22.63[a,b]	29.01[b]	20.80[a]	2.54
Methane	18.84[a]	21.06[a,b]	26.69[b]	19.41[a]	2.34
Carbon dioxide	12.66[a]	13.94[a]	16.48[a]	12.40[a]	5.88

[1] Diet: 2 = 2g starch/kg BW/d; 4 = 4g starch/kg BW/d; 6 = 6g starch/kg BW/d; 8 = 8g starch/kg BW/d.
[a-b] Means within a row with different superscripts differ ($P < 0.05$).

In Vitro Dry Matter Digestibility

There were minimal changes to the IVDMD with relation to diet during this experiment ($P < 0.90$). Both treatment period and individual animal, however, had significant effects ($P < 0.03$ and $P < 0.03$, respectively) on the IVDMD of each sample. The highest average IVDMD was detected in horses fed Diet 8 (85%). Diet 2 had an IVDMD of 84% while Diets 4 and 6 both had an IVDMD of 83%. Because the samples used included alfalfa and cecal digesta, these values can only be used to indicate changes to IVDMD. The higher digestibility of Diet 8 is consistent with previous research by Drogoul et al. (2001) and Medina et al. (2002) which found that the presence of low levels of starch in the cecum could improve digestibility in horses.

CHAPTER IV

CONCLUSIONS

This study found that even when starch consumption is lower than 2 g starch/kg BW/meal, the level which was previously stated to cause starch overload in the small intestine (Meyer et al., 1995), it is still possible for starch to reach the hindgut and influence the cecal environment. The horses in the current study consumed, at most, an average of 1.60 g starch/kg BW/meal and still observed changes to the cecal environment.

The pH in the cecum of the horses in this study decreased as the offered amount of starch in the diet increased, indicating an increase in acid production by the cecal bacteria. The number of total anaerobic bacteria and the lactic acid bacteria in this study changed very little, and did not appear to respond to the changes in diet by increasing in populations as previous literature indicated they would (Kern et al., 1973; Julliand et al., 2001; Medina et al., 2002). However, changes to the products of fermentation, such as increases in volatile fatty acid production, indicates that increasing amounts of starch were fermented by bacteria in the cecum.

In conclusion, diets with less than 2 g starch/kg BW/meal are still capable of having undegraded starch reach the hindgut and influence the cecal environment of the horse. This amount of starch is capable of increasing volatile fatty acid production, and therefore providing energy constiuents to the horse without causing detrimental decrease to pH that may result in subclinical acidosis or laminitis. Further research is required to

determine what the level of dietary starch causes changes to the microbial populations in the cecum and further decreases to the pH in the cecum.

LITERATURE CITED

Al Jassim, R.A.F., P.T. Scott, A.L. Trebbin, D. Trott and C.C. Pollitt. 2005. The genetic diversity of lactic acid producing bacteria in the equine gastrointestinal tract. FEMS Microbiol. Letters. 248:75-81.

Alexander, F. 1952. Some functions of the large intestine of the horse. Q. J. Exp. Physiol. Cogn. Med. Sci. 37:205-214.

Alexander, F. and M.E. Davies. 1963. Production and fermentation of lactate by bacteria in the alimentary canal of the horse and pig. J. Comp. Path. 73:1-8.

Anderson, R.C., G.E. Carstens, R.K. Miller, T.R. Callaway, C.L. Schultz, T.S. Edrington, R.B. Harvey and D.J. Nisbet. 2006. Effect of oral nitroethane and 2-nitropropanol administration on methane-producing activity and volatile fatty acid production in the ovine rumen. J. Bio. Tech. 97:2421-2426.

Annison, E.F. and D. Lewis. 1959. Metabolism in the Rumen. Methuen, London.

Argenzio, R.A., J.E. Lowe, D.W. Pickard and C.E. Stevens. 1974a. Digesta passage and water exchange in the equine large intestine. Am. J. Phys. 226:1035-1042.

Argenzio, R.A., M. Southworth and C.E. Stevens. 1974b. Sites of organic acid production and absorption in the equine gastrointestinal tract. Am. J. Phys. 226:1043-1050.

Argenzio, R.A. 1975. Functions of the equine large intestine and their interrelationship in disease. Cornell Vet. 65:301-331.

Arnold, F.F., G.D. Potter, J.L. Kreider, G.T. Schelling and W.L. Jenkins. 1981. Carbohydrate digestion in the small and large intestine of the equine. Pages 19-22 in: Proc. 7th Equine Nutr. Physiol. Symp., Warrenton, VA

Bailey, S.R., A. Rycroft, and J. Elliott. 2002. Production of amines in equine cecal contents in an in vitro model of carbohydrate overload. J. Anim. Sci. 80:2656-2662.

Bailey, S.R., M.-L. Baillon, A.N. Rycroft, P.A. Harris and J. Elliot. 2003. Identification of equine cecal bacteria producing amines in an in vitro model of carbohydrate overload. Appl. Environ. Microbiol. 69:2087-2093.

Bauman, H.E. and E.M. Foster. 1956. Characteristics of organisms isolated from the rumen of cows fed high and low roughage rations. J. Bacteriol. 71:333-338.

Beuvink, J.M.W. and S.F. Spoelstra. 1992. Interactions between substrate, fermentation end-products, buffering systems and gas production upon fermentation of different carbohydrates by mixed rumen microorganisms in vitro. Appl. Microbiol. Biotech. 37:505-509.

Bonhomme-Florentin, A. 1988. Degradation of hemicellulose and pectin by horse caecum contents. Br. J. Nutr. 60:185-1992

Bryant, M.P. and L.A. Burkey. 1953. Numbers and some predominant groups of bacteria in the rumen of cows fed different rations. J. Dairy. Sci. 36:218-224.

Chalupa, W. 1977. Manipulating rumen fermentation. J. Anim. Sci. 46:585-599.

Chaney, A.L. and E.P. Marbach. 1962. Modified reagents for determination of urea and ammonia. Clin. Chem. 8:130-132.

Clarke, L.L., M.C. Roberts and R.A. Argenzio. 1990. Feeding and digestive problems in horses. Physiologic responses to a concentrate meal. Vet. Clin. North Am. Equine Pract. 6:433-450.

Daly, K., C.S. Stewart, H.J. Flint and S.P. Shirazi-Beechey. 2001. Bacterial diversity within the equine large intestine as revealed by molecular analysis of cloned 16S rRNA genes. FEMS Microbiol. Ecol. 38:141-151.

Davies, M.E. 1964. Cellulolytic bacteria isolated from the large intestine of the horse. J. Appl. Bacteriol. 27:373-378

de Fombelle, A., V. Julliand, C. Drogoul and E. Jacotot. 2001. Feeding and microbial disorders in horses: 1- Effects of an abrupt incorporation of two levels of barley in a hay diet on microbial profile and activities. J. Equine Vet. Sci. 21:439-444.

Dehority, B.A. and H.W. Scott. 1967. Extent of cellulose and hemicellulose digestion in various forages by pure cultures of rumen bacteria. J. Dairy Sci. 50:1136-1141.

Doerner, K.C. and B.A. White. 1990. Assessment of the endo-1,4-beta-glucanase components of *Ruminococcus flavefaciens* FD-1. Appl. Environ. Microbiol. 56:1844-1850.

Ellis, A.D. and J. Hill. 2005. Nutritional Physiology of the Horse. Nottingham University Press, Nottingham, England.

Forsberg, C.W. 1997. Polysaccharide degradation in the rumen and large intestine. Pges 319-360 in: Gastrointestinal Microbiology Volume 1: Gastrointestinal Ecosystems and Fermentations. Chapman & Hall, New York.

Frape, D.L. 2004. Equine Nutrition and Feeding. Blackwell Publishing, Ames, IA.

Gardner, R.M., K.C. Doerner, and B.A. White. 1987. Purification and characterization of an exo-beta-1,5-glucanase from *Ruminococcus flavefaciens* FD-1. J. Bacteriol. 169:4581-4588.

Garner, H.E. J.R. Coffman, A.W. Hahn, D.P. Hutcheson and M.E. Tumbleson. 1975. Equine laminitis of alimentary origin: An experimental model. Am. J. Vet. Res. 36-441-444.

Garner, H.E., J.N. Moore, J.H. Johnson, L. Clarke, J.F. Amend, L.G. Tritschler and J.R. Coffmann. 1978. Changes in caecal flora associated with the onset of laminitis. Equine Vet. J. 10:249-252.

Ghorbani, G.R., D.P. Morgavi, K.A. Beauchemin and J.A.Z. Leedle. 2002. Effects of bacterial direct-fed microbials on ruminal fermentation, blood variables, and the microbial populations of feedlot cattle. J. Anim. Sci. 80:1977-1985.

Goodson, J., W.J. Tyznik, J.H. Cline and B.A. Dehority. 1988. Effects of an abrupt diet change from hay to concentrate on microbial numbers and physical environment in the cecum of the pony. Appl. Environ. Microbiol. 54:1946-1950.

Gorleau, D. and C.W. Forsberg. 1981. Cellulolytic activity of the rumen bacterium *Bacteroides succinogenes*. Can. J. Microbiol. 27:517-530.

Grubb, J.A. and B.A. Dehority. 1975. Effects of an abrupt change in ration from all roughage to high concentrate upon rumen microbial numbers in sheep. Appl. Microbiol. 30:404-412.

Hintz, H.F., R.A. Argenzio and H.F. Schryver. 1971. Digestion coefficients, blood glucose levels and molar percentage of volatile acids in intestinal fluid of ponies fed varying forage-grain ratios. J. Anim. Sci. 33:992-995.

Hirose, Y., S. Suzuki, Y. Asahida. 1957. Japan J. Zootech. Sci. 28:177-180.

Hungate, R.A. The anaerobic mesophilic cellulolytic bacteria. 1950. Microbiol. Mol. Biol. Rev. 14:1-49.

Hungate, R.E., R.W. Dougherty, M.P. Bryant and R.M. Cello. 1952. Microbiological and physiological changes associated with acute indigestion in sheep. Cornell Vet. 42:423-449.

Hungate, R.E. 1966. The Rumen and its Microbes. Academic Press, New York.

Julliand, V., A. de Fombelle, C. Drogoul and E. Jacotot. 2001. Feeding and microbial disorders in horses: Part 3 - Effects of three hay:grain ratios on microbial profile and activities. J. Equine Vet. Sci. 21:543-546.

Julliand, V., A. de Vaux, L. Millet and G. Fonty. 1999. Identification of *Ruminococcus flavefaciens* as predominant cellulolytic bacterial species of the equine cecum. Appl. Environ. Microbiol. 65:3738-3741.

Kern, D.L., L.L. Slyter, J.M. Weaver, E.C. Leffel and G. Samuelson. 1973. Pony cecum vs. steer rumen: The effect of oats and hay on the microbial ecosystem. J. Anim. Sci. 37:463-469.

Kern, D.L., L.L. Slyter, E.C. Leffel, J.M. Weaver and R.R. Oltjen. 1974. Ponies vs. steers: Microbial and chemical characteristics of intestinal ingesta. J. Anim. Sci. 38:559-564.

Lana, R.P., J.B. Russell and M.E. Van Amburgh. 1998. The role of pH in regulating ruminal methane and ammonia production. J. Anim. Sci. 76:2190-2196.

Latham, M.J., M.E. Sharpe and J.D. Sutton. 1971. The microbial flora of the rumen of cows fed hay and high cereal rations and its relationship to the rumen fermentation. J. Appl. Bact. 34:425-434.

Leedle, J.A.Z., M.P. Bryant and R.B. Hespell. 1982. Diurnal variations in bacterial numbers and fluid parameters in ruminal contents of animals fed low- or high-forage diets. Appl. Environ. Microbiol. 44:402-412.

Leek., B.F. 1977. Digestion in the ruminant stomach. Pages 438-474 in: Dukes' Physiology of Domestic Animals. Comstock Pub. Associates, Ithaca, NY.

Lin, C. and D.A. Stahl. 1995. Taxon-specific probes for cellulolytic genus *Fibrobacter* reveal abundant and novel equine-associated populations. Appl. Environ. Microbiol. 61:1348-1351.

Mackie, R.I. and C.A. Wilkins. 1988. Enumeration of anaerobic bacterial microflora of the equine gastrointestinal tract. Appl. Environ. Microbiol. 54:2155-2160.

Maczulak, A.E., K.A. Dawson and J.P. Baker. 1985. Nitrogen utilization in bacterial isolate from the equine cecum. Appl. Environ. Microbiol. 50:1439-1443.

Maki, I.R. and E.M. Foster. 1957. Effect of roughage in the bovine ration on types of bacteria in the rumen. J. Dairy. Sci. 40:905-913.

Mangels, J.I. and B.P. Douglas. 1989. Comparison of four commercial brucella agar media for growth of anaerobic organisms. J. Clin. Microbiol. 27:2268-2271.

McDaniel, A.L., S.A. Martin, J.S. McCann and A.H. Parks. 1993. Effects of *Aspergillus oryzae* fermentation extract on in vitro equine cecal fermentation. J. Anim. Sci. 72:2164-2172.

Medina, B., I.D. Girard, E. Jacotot and V. Julliand. 2002. Effect of a preparation of *Saccharomyces cerevisiae* on microbial profiles and fermentation patterns in the large intestine of horses fed a high fiber or high starch diet. J. Anim. Sci. 80:2600-2609.

Meyer, H., S. Radicke, E. Kienzle, S. Wilke and D. Kleffken. 1993. Investigations of preileal digestion of oats, corn, and barley starch in relation to grain processing. Pages 92-97 in: Proc. 13th Equine Nutr. Physiol. Symp. University of Florida, Gainesville, FL.

Miller, T.L. and M.J. Wolin. 1973. Formation of hydrogen and formate by *Ruminococcus albus*. J. Bacteriol. 116:836-846.

Miller, T.L. 1978. The pathway of formation of acetate and succinate from pyruvate by *Bacteroides succinogenes*. Arch. Microbiol. 117:145-152.

Miyaji, M. K. Ueda, H. Nakatsuji, T. Tomioka, Y. Kobayashi and H. Hata. 2008. Mean retention time of digesta in the different segments of the equine hindgut. Jap. Anim. Sci. J. 79:89-96.

Montgomery, L. B. Flesher and D. Stahl. 1988. Transfer of *Bacteroides succinogenes* (Hungate) to *Fibrobacter* gen. nov. as *Fibrobacter succinogenes* comb. nov. and description of *Fibrobacter intestinalis* sp. nov. Int. J. Syst. Bacteriol. 38:430-435.

Moore, B.E. and B.A. Dehority. 1993. Effects of diet and hindgut defaunation on diet digestibility and microbial concentrations in the cecum and colon of the horse. J. Anim. Sci. 71:3350-3358.

Pettipher, G.L. and M.J. Latham. 1979. Characteristics of enzymes produced by *Ruminococcus flavefaciens* which degrade plant cell walls. J. Gen. Microbiol. 110:21-27.

Pol, A. and D.I. Denmeyer. 1988. Fermentation of methanol in the sheep rumen. Appl. Environ. Microbiol. 54:832-834.

Pond, W.G., D.C. Church, K.R. Pond. 1995. Basic animal nutrition and feeding. Wiley, New York.

Potter, G.D., F.F. Arnold, D.D. Householder, D.H. Hanson and K.M. Brown. 1992. Digestion of starch in the small or large intestine of the equine. Pferdeheilkunde 1:107-111.

Prins, R.A. 1977. Biochemical activities of gut micro-organisms. Pages 73-183 in: Microbial Ecology of the Gut. Academic Press, New York.

Radicke, S., E. Kienzle and H. Meyer. 1991. Preileal apparent digestibility of oats and corn starch and consequences for cecal metabolism. Pages 43-48 in: Proc. 12th Equine Nutr. Physiol. Symp. Savoy, IL.

Reitnour, C.M. and R.L. Salsbury. 1972. Digestion and utilization of cecally infused protein by the equine. J. Anim. Sci. 35:1190-1193.

Respondek, F., A.G. Goachet and V. Julliand. 2008. Effects of dietary short-chain fructooligosaccharides on the intestinal microflora of horses subjected to a sudden change in diet. J. Anim. Sci. 86:316-323.

Rumsey, T.S., P.A. Putnam, J. Bond and R.R. Oltjen. 1970. Influence of level and type and diet on ruminal pH and VFA, respiratory rate and EKG patterns of steers. J. Anim. Sci. 31:608-616.

Sellers, A.F. and Lowe J.F. 1986. Review of large intestinal motility and mechanisms of impaction in the horse. Equine Vet. J. 18:261-263.

Slyter, L.L., R.R. Oltjen, D.L. Kern and F.C. Blank. 1970. Influence of type and level of grain and diethlstilbestrol on rumen microbial populations of steers fed all-concentrate diets. J. Anim. Sci. 31:996-1002.

Stainer, R.Y., J.L. Ingraham, M.L. Wheelis, P.R. Painter. 1986. The Microbial World: 5th Edition. Prentice-Hall. Englewood Cliffs, NJ.

Stewart, C.S. and M.P. Bryant. 1988. The rumen bacteria. Pages 21-75 in: The Rumen Microbial Ecosystem. Springer, New York. P 21-75

Thomas, T.D., K.W. Turner and V.L. Crow. 1980. Galactose fermentation by *Streptococcus lactis* and *Streptococcus cremoris*: Pathways, products, and regulation. J. Bacteriol. 144:672-682.

Tiley, J.M.A. and R.A. Terry. 1963. Two-stage technique for the in vitro digestion of forage crops. J. Br. Grassl. Soc. 18:104-111.

Vanzant, E.S. and R.C. Cochran. 1994. Performance and forage utilization by beef cattle receiving increasing amounts of alfalfa hay as a supplement to low-quality tallgrass-prairie forage. J. Anim. Sci. 72:1059-1067.

Wallace, R.J., R. Onodera and M.A. Cotta. 1997. Metabolism of nitrogen-containing compounds. Pages 217-249 in: The Rumen Microbial Ecosystem. Springer, New York.

Wilkins, P.A. and Lowe, J.E. 1993. Technique for long-term right dorsal colon fistulization in ponies. Am. J. Vet. Res. 54:183-184.

Willard, J.G., J.C. Willard, S.A. Wolfram and J.P. Baker. 1977. Effect of diet on cecal pH and feeding behavior of horses. J. Anim. Sci. 45:87-93.

Wolin, M.J. 1960. A theoretical rumen fermentation balance. J. Dairy. Sci. 43:1452-1459.

Wright, D.E. and R.E. Hungate. 1967. Amino acid concentrations in rumen fluid. Appl. Environ. Microbiol. 15:148-151.

Wysocki A.A. and J.P. Baker. 1975. Utilization of bacterial protein from the lower gut of the equine. Pages 21-42 in: Proc: 4th Equine Nutr. Physiol. Symp. University of California Davis, Pomona, CA.

VITA

Name:	Kristen Leigh Wilson
Address:	16100 Georgia Peach Ave Covington, GA 30014
Email Address:	kwilson283@gmail.com
Education:	B.S., Animal and Dairy Science, University of Georgia, 2005 M.S., Animal Science, Texas A&M University, 2009